Investigating the global and local dynamics of homotetrameric enzyme pteridine reductase by molecular dynamics and enhanced sampling simulations

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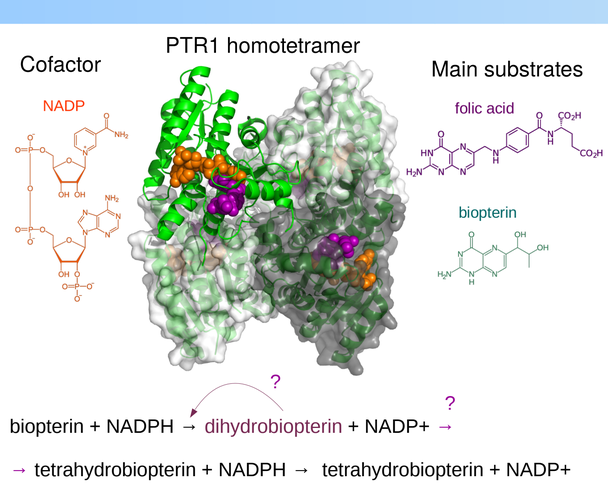
Pteridine reductase 1 - questions about the enzymatic mechanism



Trypanosomatid parasites are a major health issue of many developing countries, as they cause **wide-spread diseases in humans**. **Folate pathway** enzyme**, pteridine reductase 1** (**PTR1,** EC 1.5.1.33), is an interesting drug target. Structural and dynamical properties of PTR1 are poorly explored.

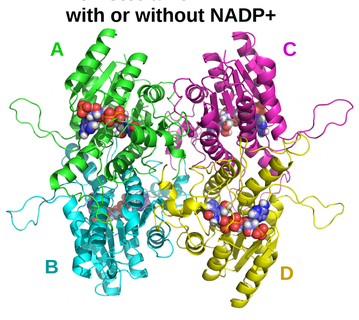
It is known that larger concentrations of semiproduct inhibit the enzymatic reaction (**substrate inhibition**).

1. Is allostery important for PTR1 enzymatic mechanism? (substrate inhibition phenomenon)
2. How tight is NADP+/NADPH binding? How is it recycled after the catalytic reaction?

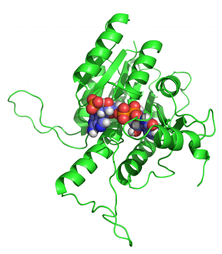
Studied systems and simulations

PTR1 variant currently considered: *Leishmania major* PTR1 (LmPTR1), PDB: 1E92.
Modeling of missing protein loops performed with Maestro (Schrödinger, LCC) and refined with ModLoop server, then inspected with WHAT_IF server (http://swift.cmbi.ru.nl).

homotetramer with or without NADP+



monomer with or without NADP+



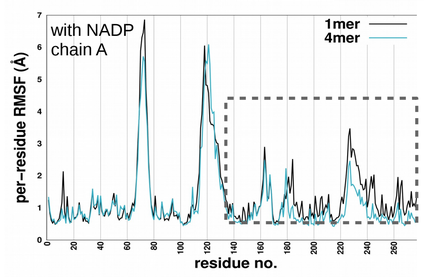
- **MD systems:** explicit water, with NaCl ionic strength of 0.1M, at 310K and protonated at pH 7. Simulations in PBC in truncated octahedral cell with 10-Å boundary.
- **Force field:** ff14sb (Maier et al., 2015) for protein, Ryde parameters for NADP+ (Holmberg et al., 1999), TIP3P water, Li/Merz 12-6 HFE set parameters for Na/Cl-+ ions (Pengfei et al., 2015).
- **System preparation:** AmberTools 16 (http://ambermd.org)
- **MD:** NAMD 2.11
- **Simulation protocol in brief:** minimization → NVT heating -> NpT equilibration -> NVT production.

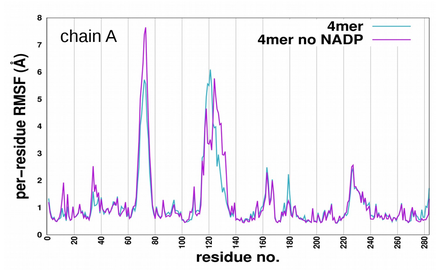
Preliminary explicit solvent MD results

150 ns of MD for each of the systems (to be extended and repeated).

Residue fluctuations are affected by the enzyme multimeric state, but not by NADP+ binding.

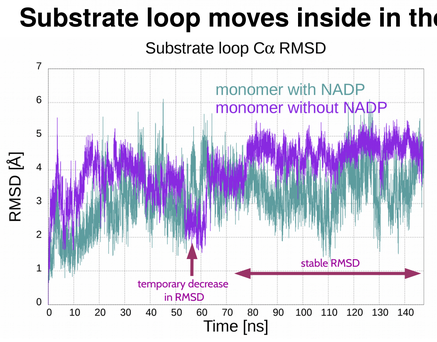
Monomer is more flexible than homotetramer in the C-terminal region.

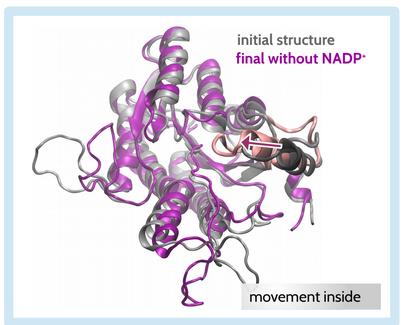


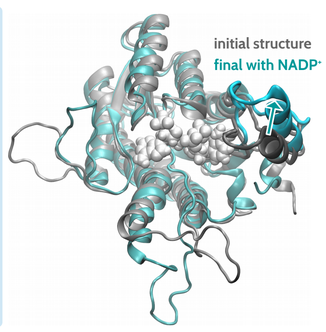


Homotetramers with and without NADP+ are comparably flexible.

Substrate loop moves inside in the monomer without NADP+ and forms stable interactions.

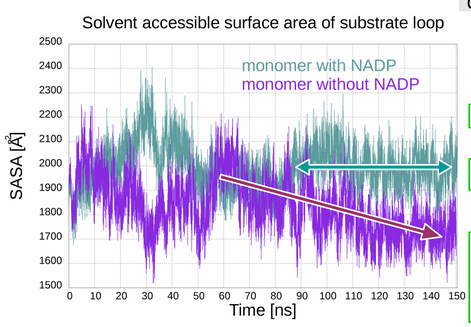


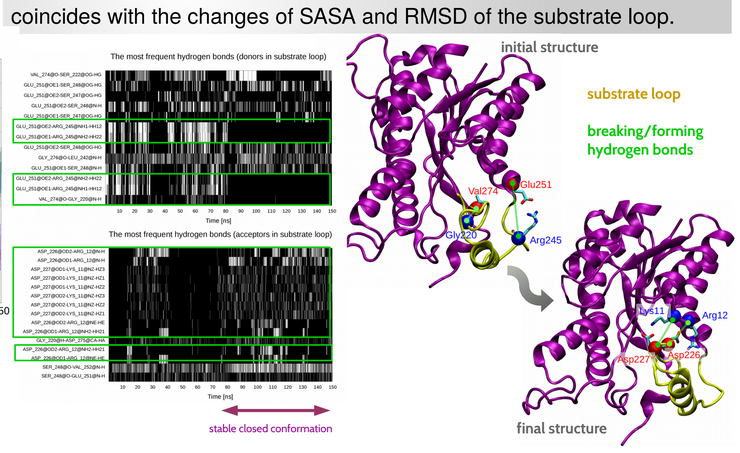




Without NADP+, substrate loop RMSD is on average higher than with NADP+.

Formation and breaking of hydrogen bonds in the variant without NADP+ coincides with the changes of SASA and RMSD of the substrate loop.





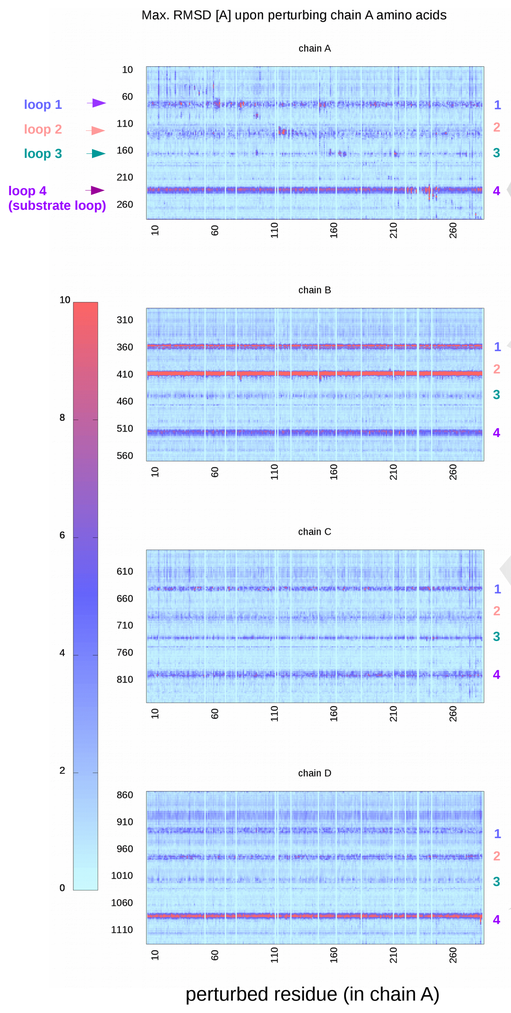
Without NADP+ SASA decreases upon loop closing.

Perturbing in the enzyme structure

Method:

- Non-equilibrium Rotamerically Induced Perturbations (RIP) method (Ho & Agard, 2011) was used to probe slow timescale motions of LmPTR1 homotetramer with NADP+.
- Amino acid side chain torsional angles are perturbed one by one: after short equilibration, perturbation pulses are applied to each residue 100 times during 10-ps of implicit solvent MD (GBSA).
- Seperate perturbation simulations are performed for each residue of chain A of the LmPTR1 homotetramer (283 amino acids excluding these that do not have rotational degrees of freedom on side chains) and their effect is analyzed on the dynamics of the remaining residues.

Global flexibility of the LmPTR1 homotetramer upon perturbations of particular residues:

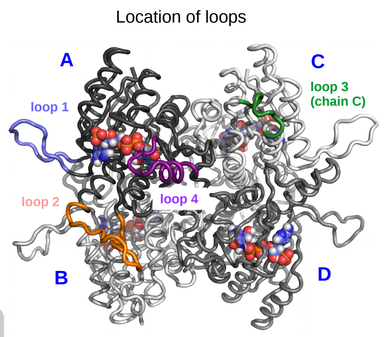


Almost all residue perturbations in chain A affect substrate loop deformations in this chain, but other loops of chain A are affected by only selected residues.

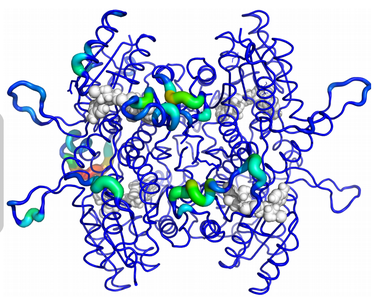
Loops 1, 2 and 4 of chain B are significantly affected by perturbations in chain A, independent of which residue is perturbed.

Perturbations in chain A induce the weakest deformations in chain C, despite a considerable interface (35 residues within 4 Å).

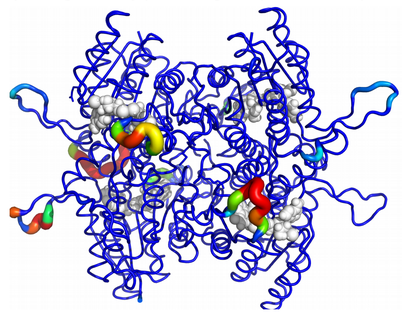
Substrate loop is affected the most in chain A and D.



The most perturbed residues (averaged Cα RMSD)



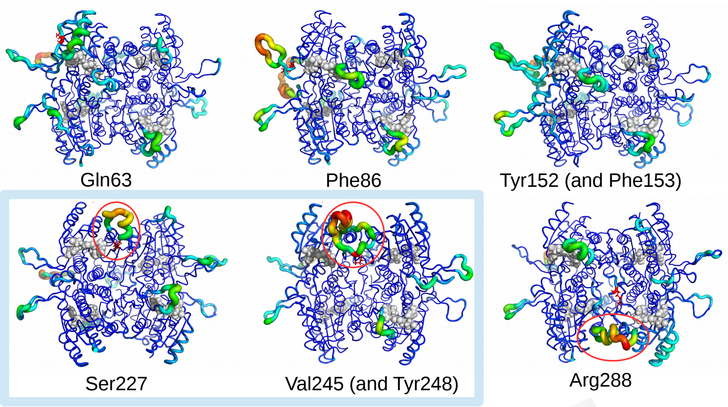
Susceptibility of a residue to large response (# of significantly perturbing RIP perturbations)



General conclusion: The loops in chains B, C, D are uniformly affected by perturbations of almost all residues in chain A. Thus, they may be important for dynamical communication between the PTR1 subunits.

The most interesting hot-spots (highly perturbing residues)

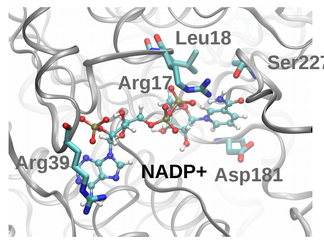
the number of significant responding residues (> 6 Å of Cα-RMSD) for each perturbed residue.



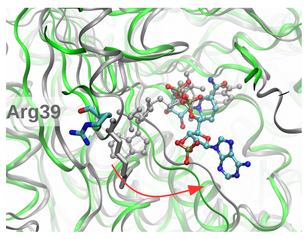
Substrate loop in chain A is significantly destabilized.

Substrate loop in chain D is significantly destabilized.

Binding of NADP+ is distinctly affected by perturbations of these residues:



Example: strong effect of perturbing Arg39



Summary and outlook

- **Substrate loop closing is observed in the PTR1 monomer without NADP+.** The movement to the closed conformation is associated with breaking initial and forming new hydrogen bonds. **Stability of the final closed conformation suggests that this conformational change may be functionally relevant, for example in the process of recycling of the NADP+ cofactor.**
- RIP method identifies stability hot-spots for the LmPTR1 homotetramer and suggests that **flexible loops may be important for long-range dynamical communication between the subunits.**
- **RIP simulations also indicated, which residues are the most critical for stability of the NADP+ cofactor in its binding cleft.**

References

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